AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph beginning on page 6, line 11, with the following amended paragraph:

~~Figure 2 shows~~ Figures 2A-2E show protein expression in the rotating wall vessel. Figures 2A-2C show ~~Figure 2A shows~~ analysis of the expression and endosomal compartmentation of megaline, and cubulin in renal cells following rotating wall vessel culture. The ability of flow cytometry to make simultaneous measurements of entrapped fluorescein dextran as an endosomal marker and antibody binding allows construction of three dimensional frequency histograms displaying entrapped fluorescein dextran fluorescence against antibody binding on horizontal axes. A control sample shows vesicles negative for fluorescein on the left and fluorescein containing endosomes on the right (2000 vesicles depicted left panel). A control without fluorescein entrapped shows only the left population (not shown). Co localization of anti-cubulin binding demonstrates that all the fluorescein positive endosomes are positive for cubulin, while non-endosomal membranes can be subdivided into cubulin positive and negative populations (middle panel). This pattern is repeated for anti-megalin binding in renal cortical cells (right panel).

Please replace the paragraph beginning on page 6, line 25, with the following amended paragraph:

Figure 2D ~~2B~~ shows quantitation of cubulin, and megalin antibody binding to renal cell membranes under various culture conditions. Analysis of protein expression in cultured cells by antibody binding used classic serial log dilution antibody curves. An increase in binding with a decrease in dilution is pathognomonic for specific anitbody binding during flow cytometry analysis. Binding of anti-cubulin antisera to membrane vesicles prepared from renal cortical cells after 16 days in culture, detected by the fluorescence of a phycoerthyrein tagged secondary antibody, shows an almost two log increase in binding with antibody dilution (upper left panel below). This increased cubulin antibody binding in the cells grown in the rotating wall vessel (STLV) is more than five times the expression seen in stirred fermentors. Similarly, there was no detectable expression in the conventional cultures resulting in a flat line (not shown). Binding of normal serum and minimal dilution of primary antisera were not detectably different. Binding curves for anti-megalin antiserum showed a similar pattern (not shown).

Please replace the paragraph beginning on page 7, line 3, with the following amended paragraph:

Figure 2E ~~2C~~ depicts non-specific (minimum) and peak binding of each antiserum following rotating wall vessel culture and two-dimensional SDS-PAGE analysis of protein content of cells following rotating wall vessel culture. Analysis of the protein content of cultures of the natural mixture of rat renal cortical cells after 16 days culture in gas permeable bags as a control (left panel) or rotating wall vessel (right panel) depicts

changes in a select set of proteins. Molecular weight (14-220 kDa) on the abscissa is displayed against isoelectric point (pH 3-10) on the ordinate.

Please replace the paragraph beginning on page 8, line 1, with the following amended paragraph:

~~Figure 4 shows~~ Figures 4A-4C show structure and effects of antisense probe for shear stress response element on rat renal cortical epithelial cells. Figure 4A shows the structure. The probe with sequence CTGAGACCGATATCGGTCTCAG (SEQ ID No:1) has two possible conformations. As a single strand it would fold back on itself to form a binding element for the transcription factor. As a double strand it would then have two binding sites for the transcription factor, one in the sense orientation and one in the antisense orientation.

Please replace the paragraph beginning on page 8, line 8, with the following amended paragraph:

Figures 4B and 4C show ~~Figure 4B shows~~ effects of antisense shear stress response to element probe on time dependent gene expression. The antisense probe added to conventional 2-dimensional cultures of rat renal cortical cells at 80 nm increases MnSOD in a time dependent manner. Comparison is made to controls with the active binding site scrambled. In contrast the probe has no effect on villin gene expression.

Please replace the paragraph beginning on line 16 of page 13 with the following amended paragraph:

To quantitate the total and endosomal expression of cubulin, megaline and aquaporin-2 cells in conventional culture, stirred fermentors and slow turning lateral vessel rotating wall vessels, 0.3 mg/ml 10S fluorescein-dextran was to each cell culture for 10 minutes at 37°C in the CO2 incubator. This loads an entrapped fluorescent dye into the early endosomal pathway (9, 47). Cells were then immediately diluted into ice cold phosphate buffered saline and washed once. Next, the cells were homogenized with 6 passes of a tight fitting glass-Teflon motor driven homogenizer. A post-nuclear supernatant was formed as the 11,000g supernatant, 180,000g pellet of membrane vessels (Figures 2A-2C).

Please replace the paragraph beginning on line 25 of page 13 with the following amended paragraph:

Aliquots of membrane vesicles were labeled with megaline or cubulin antisera. The megalin and cubulin antisera were rabbit polyclonals raised to affinity purified and chromatographically pure receptor (43, 48). Membrane vesicles were first pre-incubated in 50% normal goat serum for 2 hours to reduce non-specific binding of secondary antisera raised in goat. After washing aliquots of membrane vesicles were stained with serial log dilution of antisera and incubated at 4°C overnight. After further washing 1:40 of goat anti-rabbit affinity purified rat pre-absorbed phycoerthyrein conjugated secondary antiserum was added, and incubated for 4 hours at room temperature. Prior to flow

cytometry the membrane vesicles were washed and resuspended in 200 mM mannitol, 100 mM KCl, 10 mM HEPES, pH 8.0 with Tris to which had been added 10 mM nigericin. In the presence of potassium, nigericin collapses pH gradients, ensuring optimal fluorescence of the highly pH dependent fluorescein-dextran emission. Fluorescein-dextran and antibody staining tagged by phycoerythrein were now analyzed and co-localized on a vesicle-by-vesicle basis by flow cytometry (Figure 2D ~~2B~~).

Please replace the following paragraph beginning on line 13 of page 15 with the following amended paragraph:

Double stranded genetic decoys matching the sequence of a known shear stress response element were synthesized (Chemicon International Inc., La Jolla, CA) (structure and sequence shown at top of ~~Figure 4~~ Figure 4A). These decoys had a terminal phosphothioate moiety to prevent intracellular lysis, and a phosphodiester backbone to facilitate passage across cell membranes (49). Passage to and accumulation in the nuclear compartment of cultured cells was confirmed by confocal imaging of a fluorescein tagged decoy. Three decoys were synthesized: the active decoy, a random sequence control in which the six bases of the shear stress response element were scrambled, and a fluorescein conjugated form of the decoy. Decoys were placed in the cell culture medium of rat renal cortical cells grown as above in conventional two-dimensional culture. Aliquots of cells exposed to control or active sequence decoy at 80nm concentration were harvested at 2, 6, and 24 hours after exposure.

Please replace the following paragraph beginning on line 11 of page 19 with the following amended paragraph:

As the endosomal pathway has been implicated to play a central role in the function and pathophysiology of cubulin and megalin, entrapped endosomal markers were co-localized with receptor antibody binding. The ability of flow cytometry to make simultaneous measurements of entrapped fluorescein dextran as an endosomal marker and antibody binding allows construction of three dimensional frequency histograms displaying entrapped fluorescein dextran fluorescence against antibody binding on horizontal axes and number of vesicles in each channel up out of the page (Figures 2A-2C). A control sample shows vesicles negative for fluorescein on the left and fluorescein containing endosomes on the right (200 vesicles depicted, left panel). A control without fluorescein entrapped shows only the left population (not shown). Co localization of anti-cubulin binding demonstrates that all the fluorescein positive endosomes were positive for cubulin, while non-endosomal membranes could be subdivided into cubulin positive and negative populations. (middle panel). This pattern was repeated for anti-megalin binding in renal cortical cells (right panel) in culture.

Please replace the paragraph beginning on line 26 of page 19 with the following amended paragraph:

Next, analysis of protein expression in cultured cells by antibody binding used classic serial log dilution antibody curves. An increase in binding with a decrease in dilution is pathognomonic for specific antibody binding during flow cytometry analysis.

Binding of anti-cubulin antisera to membrane vesicles prepared from renal cortical cells after 16 days in culture, detected by the fluorescence of a phycoerthyrein tagged secondary antibody, shows an almost two log increase in binding with antibody dilution ~~(Figure 2B)~~ (Figure 2D). This increase in cells grown in the rotating wall vessel (slow turning lateral vessel) is more than five times the expression seen in stirred fermentors. Similarly there was no detectable expression in the conventional cultures resulting in a flat line (not shown). Comparison of maximal binding of the anti-cubulin antibody to minimum taken to be the antibody dilution at which there is no further decline in signal with primary antibody dilution is shown in ~~Figure 2C~~ Figure 2E. Binding of normal serum and minimal dilution of primary antisera were not detectably different. Binding curves for anti-megalin antiserum showed a similar pattern (not shown) but the peak binding was a little lower ~~(Figure 2C)~~ (Figure 2E). Again stirred fermentor has much less expression than the rotating wall vessel (slow turning lateral vessel) and the conventional cell membranes have no detectable binding (not shown).

Please replace the paragraph beginning on line 15 of page 20 with the following amended paragraph:

To examine the proportion of proteins changing in the rotating wall vessel, two-dimensional gel SDS-PAGE analysis on cultures grown in the rotating wall vessel and bag controls were performed ~~(Figure 2d)~~. The results ~~shown in Figure 2D~~ demonstrate[s] changes were in a selected group of proteins.